Long-term Intake of *trans*-Fatty Acids and Risk of Gallstone Disease in Men

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Background: The consumption of *trans*-fatty acids adversely affects blood lipid levels. The relationship with the incidence of gallstone disease is unknown.

Methods: We prospectively studied consumption of *trans*-fatty acids in relation to the risk of gallstone disease in a cohort of 45 912 men. *trans*-Fatty acid consumption was assessed using a validated semiquantitative food frequency questionnaire. Newly diagnosed gallstone disease, by radiology or cholecystectomy, was ascertained biennially.

Results: During 14 years of follow-up, we documented 2356 new cases of symptomatic gallstones. After adjusting for age and other potential risk factors, we found that compared with men in the lowest quintile of dietary intake of *trans*-fatty acids, the relative risk (RR) of gallstone disease for those in the highest quintile was 1.23 (95% confidence interval [CI], 1.04-1.44; *P* for trend, .03). Among individual *trans*-fatty acids, the RR for *trans*-oleic fatty acid, when extreme quintiles were compared, was 1.24 (95% CI, 1.06-1.45; *P* for trend, .02). Intakes of *trans*-palmitoleic fatty acid (RR, 1.09; 95% CI, 0.90-1.31), *trans,trans* 18:2 fatty acid (RR, 1.14; 95% CI, 0.96-1.34), and *cis-trans* 18:2 fatty acid (RR, 1.00; 95% CI, 0.86-1.16) were not significantly associated with the risk.

Conclusions: Our results suggest that a higher intake of *trans*-fatty acids modestly increases risk of gallstone disease. This adds to the concern that partial hydrogenation of vegetable oils to form shortening and margarine can lead to adverse health effects.

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GALLSTONE DISEASE IS COMmon in the United States and other Western countries and is increasingly a major cause of digestive morbidity leading to hospital admission.[1,2] Among Western populations, approximately 80% of the gallstones were cholesterol stones.[3] Many factors have been associated with risk of cholesterol gallstones, but hypersecretion of cholesterol into the biliary tree is an important determinant of gallstone formation.[3] High plasma triglyceride levels and low plasma high-density lipoprotein cholesterol (HDL-C) are associated with a greater risk of cholesterol gallstone disease.[3,4]

trans-Isomers of fatty acids are formed during the process of partial hydrogenation, when liquid vegetable oils are converted to margarine and shortening and contribute to the hardness of the products. Many people use these processed vegetable fats instead of animal fats containing saturated fat and cholesterol because of health concerns. The amounts of *trans*-fatty acids consumed is presently estimated to account for an average of 2% to 5% of total energy intake and approximately 5% of total fat in the United States[5]; the figure varies, depending on the food sources. Changes in the fat composition of the diet can have major effects on physiologic mechanisms because the number and position of double bonds influence the function and metabolism of fatty acids. Studies of the effects of *trans*-fatty acids on serum lipid levels have been inconclusive.[6-8] Some studies show that *trans*-fatty acids can reduce plasma concentrations of HDL-C and increase low-density lipoprotein cholesterol (LDL-C) and triglycerides relative to the parent natural fat.[9-11]

There has been concern for years that high intake of *trans*-fatty acids could have adverse health effects because they are structurally similar to saturated fats and lack the essential metabolic activity of the parent compounds.[12-15] Although a high intake of *trans*-fatty acids may cause a deranged lipid profile that is associated with gallstone formation, epidemiologic studies on the relationship between consump-

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tion of *trans*-fatty acids and risk for gallstones are few, and the effects of individual *trans*-isomers on the incidence of gallstone disease are not known.

To address these issues, we examined long-term intakes of total *trans*-fatty acids and individual *trans*-isomers in relation to the occurrence of gallstone disease in a large cohort of US men.

METHODS

STUDY POPULATION

The Health Professionals Follow-up Study began in 1986, when 51 529 US male dentists (58%), veterinarians (20%), optometrists (7%), osteopathic physicians (4%), and podiatrists (3%) (age, 40-75 years) returned a questionnaire by mail regarding diet, medications, and medical history. Follow-up questionnaires have been sent every 2 years to update information on exposures and to ascertain the occurrence of newly diagnosed illnesses, including gallstone disease. Diet was assessed in 1986, 1990, 1994, and 1998. At baseline, we excluded men who reported a cholecystectomy or a diagnosis of gallstone disease before 1986; men with a diagnosis of cancer before 1986; men with a reported daily energy intake outside the range of 800 to 4200 kcal/d; and men with 70 or more blank food items on the dietary questionnaire. After exclusions, the study population comprised 45 912 men who were followed up from 1986 to 2000. The average follow-up rate for biennial questionnaires was greater than 94% in each 2-year follow-up cycle.

ASSESSMENT OF DIET

Dietary information was derived from a 131-item semiquantitative food frequency questionnaire (SFFQ).[16] Participants were asked to indicate the frequency, on average, of consuming a typical serving size of selected foods during the previous year. There were 9 options for respondents to choose from, ranging from never or less than once per month to 6 or more times per day. We asked about the type of fat ordinarily used for baking and frying food and at the table. Participants were asked to write in the brand and type of cooking oil and form of margarine usually used (stick or tub). Distinction of the type of margarine is important because stick margarine has a higher proportion of *trans*-isomers. Composition values for total *trans*-isomer contents of foods were based on analyses by Enig and colleagues[17] and Slover and colleagues.[18] In these calculations, we included all *trans*-isomers of carbon-18 fatty acids, and we assumed an average *trans*-isomer content equal to 32.5% of total fat for stick margarine and 17.5% of total fat for tub margarine. The intake of *trans*-fatty acids and of other nutrients was calculated by multiplying the consumption frequency of each unit of food by the nutrient content of the specified portions. Data for other dietary variables were obtained mainly from US Department of Agriculture sources.[19] To assess the validity of our measure of *trans*-fatty acid intake, the calculated intake of *trans*-fatty acids was compared with its concentration in subcutaneous adipose tissue by gas-liquid chromatography. Two studies were conducted: one among 115 women selected as controls in a case-control study of breast cancer[20] and one among 118 male participants in a cohort study.[21] In both investigations, *trans*-isomer intake was estimated using a version of the food frequency questionnaire adopted in the present study. *trans*-Isomers constituted 4.4% of fatty acids in subcutaneous adipose tissue and 5.8% of fatty acids calculated from the dietary questionnaire among women; comparable values were 4.2% and 5.4% among men. The Spearman rank correlation between calculated intake as a proportion of fat and the proportion in subcutaneous adipose tissue was 0.51 among women ($P<.001$) and 0.34 among men ($P<.001$). To assess the reproducibility of the measurement of *trans*-fatty acid intake, the same group of men was asked to complete a second food frequency questionnaire 1 year after the first; the correlation between the 2 measurements was 0.63. At this institution (Channing Laboratory, Department of Medicine, Harvard Medical School, Boston, Mass), the calculation and validity of *trans*-fatty acid intake and its health effects have been reported in other studies.[22,23] A full description of the SFFQ and the procedures used for calculating nutrient intake, as well as data on reproducibility and validity in this cohort, were reported previously.[24] The validity of the SFFQ was assessed in a random sample of 127 participants living in the Boston area. All nutrients were adjusted for total energy intake using regression analysis. This adjustment for total energy intake is analogous to the isocaloric conditions used in feeding experiments to assess the effects of specific nutrients. This approach is based on the concept that the composition of the diet, independent of total energy intake, is the most relevant to dietary recommendations.[24]

ASCERTAINMENT OF END POINTS

The primary end point was incident symptomatic gallstones. In 1986 and on each follow-up questionnaire, participants were asked whether they had undergone a cholecystectomy or had been diagnosed as having gallstones by a physician. Participants were also asked whether the gallstone diagnosis had been confirmed by radiographic procedures or surgery and whether their gallstones were symptomatic. To verify the self-reports of gallstone disease, a random sample of 441 medical records of participants who reported a cholecystectomy or gallstones were reviewed and the diagnosis was confirmed in nearly all (99%) of these. Moreover, we confirmed all but one of the self-reported diagnostic procedures by medical record review.

STATISTICAL ANALYSIS

For each participant, follow-up time accrued from the month of return of the 1986 questionnaire and ended at the month of cholecystectomy, diagnosis of symptomatic gallstones, death, or the end of the study period, whichever occurred first. Men with asymptomatic gallstones or those whose gallstone diagnosis was not based on radiology or surgery and men with diagnosed cancer were excluded from subsequent follow-up. Thus, the eligible population at risk comprised only those who remained free of gallstone disease and cancer at the beginning of each 2-year follow-up interval. Incidence rates were calculated by dividing the number of events by person-years of follow up in each category. Relative risks (RRs) were calculated as the incidence rate of gallstone disease among men in different categories of *trans*-fat intake compared with the incidence rate among men in the lowest intake category, with adjustment for age in 5-year categories. The incidence of gallstone disease was examined in relation to the cumulative average of exposure variables from all available questionnaires up to the start of each 2-year follow-up interval, using methods for repeated measurement.[25] Age-adjusted RRs were calculated using the Mantel-Haenszel summary estimator.[26] Multivariate RRs were computed using the Cox proportional hazards regression model.[27] In multivariate analyses, we simultaneously included intake of total energy and potential confounding covariates, including age, body mass index (calculated as weight in kilograms divided by the square of height in meters), weight change during the past 2 years, cigarette smoking, history of diabetes mellitus, intakes of alcohol, caffeine, and dietary fi-

Table 1. Baseline Characteristics of 45 912 US Men According to Quintile of Energy-Adjusted *trans*-Fat Intake in 1986 in the Health Professionals Follow-up Study

	Quintiles of *trans*-Fat				
Characteristic*	1 (Lowest)	2	3	4	5 (Highest)
No. of participants	9265	9091	9257	9079	9220
trans-Fat intake, mean, g/d	1.4	2.2	2.7	3.3	4.5
Age, mean, y	53.6	53.4	53.3	53.3	53.4
BMI	24.3	24.9	25.2	25.1	25.0
Physical activity, METs	26.2	21.2	19.2	17.5	15.8
Current smoker, %	6.3	8.7	9.9	11.0	10.7
Mean daily intake					
Total energy, kcal	1971	2003	2014	2003	1956
Carbohydrate, g	258	237	230	225	224
Protein, g	96	94	93	91	87
Alcohol, g	14	13	12	10	7
Caffeine, mg	190	230	243	263	283
Saturated fat, g	19	23	25	27	28
Polyunsaturated fat, g	12	13	13	13	14
Monounsaturated fat, g	21	26	28	30	32
Dietary fiber, g	26	22	20	19	18

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); METs, metabolic equivalent tasks per week (defined as the energy consumed per minute of sitting at rest).
*Values have been standardized for age of the cohort.

ber, physical activity, thiazide diuretics, nonsteroidal anti-inflammatory drugs, saturated fat, and polyunsaturated and monounsaturated fats. Tests of linear trend across increasing categories of dietary *trans*-fat were conducted by assigning the median intake of *trans*-fat for categories and treating these as a single continuous variable. All RRs are presented with 95% confidence intervals (CIs), and all reported *P* values are 2-sided. All analyses were performed with Statistical Analysis System software, release 8.2 (SAS Institute Inc, Cary, NC).

RESULTS

At baseline in 1986, the median intakes of *trans*-fatty acids for the highest and lowest quintiles varied nearly 3-fold (**Table 1**). Intake of *trans*-fat was positively correlated with intake of saturated fat ($r=0.51$), monounsaturated fat ($r=0.60$), and polyunsaturated fat ($r=0.15$). Men with a higher intake of *trans*-fatty acids consumed less carbohydrate, protein, and fiber but had higher intakes of coffee, polyunsaturated fat, monounsaturated fat, and saturated fat. Men who reported a higher *trans*-fatty acid intake tended to be more sedentary and to drink less alcohol.

During 546 112 person-years of follow-up from 1986 to 2000, we documented 2356 incident cases of symptomatic gallstones, of which 1294 cases required cholecystectomy. Because intake of *trans*-fatty acids was associated both directly and inversely with several potential risk factors, we analyzed their relations with gallstone disease before and after adjustment for these variables.

The RR for men consuming *trans*-fatty acids in the highest quintile compared with men in the lowest quintile was 1.29 (95% CI, 1.13-1.47; *P* for trend, <.001) in age-adjusted analysis (**Table 2**). The association remained significant but was slightly attenuated (RR, 1.23; 95% CI, 1.04-1.44; *P* for trend, .03) after adjusting for multiple potential confounding variables, including age, body mass index, recent weight change, cigarette smoking, history of diabetes mellitus, intakes of alcohol, caffeine, and dietary fiber, physical activity, thiazide diuretics, nonsteroidal anti-inflammatory drugs, saturated fat, and polyunsaturated and monounsaturated fats, when extreme quintiles were compared (Table 2).

We further examined the associations of major specific types of *trans*-fatty acids with risk of gallstone disease. *trans*-Oleic fatty acid was significantly associated with an increased risk. The RR for men consuming *trans*-oleic fatty acid in the highest quintile compared with men in the lowest quintile was 1.29 (95% CI, 1.13-1.48; *P* for trend, <.001) in the age-adjusted analysis. After adjusting for multiple potential confounding variables, the RR remained significant but was slightly attenuated (RR, 1.24; 95% CI, 1.06-1.45; *P* for trend, .02). Intakes of *trans*-palmitoleic fatty acid (RR, 1.09; 95% CI, 0.90-1.31; *P* for trend, .38), *trans,trans* 18:2 fatty acid (RR,1.14; 95% CI, 0.96-1.34; *P* for trend, .25), and *cis-trans* 18:2 fatty acid (RR, 1.00; 95% CI, 0.86-1.16; *P* for trend, .96) were not significantly associated with the risk of gallstone disease in the multivariate analyses.

To examine the possibility that latent gallstone symptoms might distort the relation, thereby biasing the results, we conducted an analysis excluding all cases that occurred during the first 4-year follow-up period. Compared with men in the lowest quintile of dietary intake of *trans*-fatty acids, men in the highest quintile had a multivariate RR of 1.20 (95% CI, 1.00-1.45; *P* for trend, .09) after excluding the first 4-year follow-up period.

We also addressed the possibility of detection bias by excluding cases with unremoved stones because these were presumably less symptomatic, limiting the analysis to cholecystectomy cases. The multivariate RR for men in the highest quintile of dietary intake of *trans*-fatty ac-

Table 2. Adjusted RRs of Gallstone Disease According to Quintiles of Intake of Energy-Adjusted Total *trans*-Fatty Acids and Various Types of *trans*-Fatty Acids Among US Men in the Health Professionals Follow-up Study, 1986-2000

Variable	Quintiles, RR (95% CI)					*P* for Trend
	1 (Lowest)	2	3	4	5 (Highest)	
Total *trans*-fatty acids						
Model 1: age adjusted	1.00 (Reference)	1.16 (1.01-1.33)	1.29 (1.33-1.48)	1.26 (1.10-1.44)	1.29 (1.13-1.47)	<.001
Model 2: multivariate*	1.00 (Reference)	1.11 (0.96-1.28)	1.24 (1.07-1.44)	1.19 (1.02-1.39)	1.23 (1.04-1.44)	.03
trans-Oleic fatty acid						
Model 1: age adjusted	1.00 (Reference)	1.18 (1.03-1.35)	1.28 (1.12-1.46)	1.24 (1.09-1.42)	1.29 (1.13-1.48)	<.001
Model 2: multivariate*	1.00 (Reference)	1.14 (0.99-1.31)	1.22 (1.05-1.41)	1.19 (1.02-1.39)	1.24 (1.06-1.45)	.02
trans-Palmitoleic fatty acid						
Model 1: age adjusted	1.00 (Reference)	1.22 (1.07-1.38)	1.15 (1.00-1.32)	1.16 (1.01-1.32)	1.35 (1.19-1.54)	<.001
Model 2: multivariate*	1.00 (Reference)	1.13 (0.98-1.31)	1.02 (0.87-1.20)	0.98 (0.83-1.17)	1.09 (0.90-1.31)	.38
trans,trans 18:2 Fatty acid						
Model 1: age adjusted	1.00 (Reference)	1.11 (0.97-1.27)	1.32 (1.16-1.51)	1.28 (1.13-1.46)	1.22 (1.07-1.40)	.002
Model 2: multivariate*	1.00 (Reference)	1.08 (0.93-1.24)	1.26 (1.08-1.46)	1.22 (1.04-1.42)	1.14 (0.96-1.34)	.25
cis-trans 18:2 Fatty acid						
Model 1: age adjusted	1.00 (Reference)	1.08 (0.95-1.23)	1.12 (0.98-1.27)	1.18 (1.03-1.34)	1.12 (0.98-1.27)	.06
Model 2: multivariate*	1.00 (Reference)	1.02 (0.89-1.17)	1.03 (0.90-1.19)	1.07 (0.92-1.23)	1.00 (0.86-1.16)	.96

Abbreviations: CI, confidence interval; RR, relative risk.
*Model 2: multivariate model included the following: age, periods of follow-up (every 2 years), body mass index, weight change during the past 2 years, physical activity, dietary fiber, diabetes, thiazide diuretics, nonsteroid anti-inflammatory drugs, pack-years of smoking, alcohol intake, caffeine intake, saturated fat, monounsaturated fat, polyunsaturated fat, and total energy intake.

ids compared with men in the lowest quintile was 1.37 (95% CI, 1.10-1.71; *P* for trend, .03). To evaluate the potential for detection bias due to an increased medical surveillance, we additionally excluded men without a routine medical check-up between 1986 and 1988. The multivariate RR for men in the highest quintile of dietary intake of *trans*-fatty acids compared with men in the lowest quintile was 1.23 (95% CI, 1.04-1.44; *P* for trend, .03).

COMMENT

In this large cohort study we observed that a higher intake of *trans*-fatty acids was associated with a higher risk of gallstone disease that was not accounted for by other potential risk factors, including other measured dietary variables. We also observed a positive relation between intake of *trans*-oleic fatty acid, the main *trans*-isomer in partially hydrogenated vegetable oils,[28] and the occurrence of gallstone disease.

Types of dietary fat can influence bile lithogenicity and cholesterol gallstone formation.[29] The mechanism by which fats alter gallstone formation has been open to question; however, a preponderance of evidence suggests that high plasma triglyceride and low HDL-C levels are independent risk factors for gallstones.[3] Cholesterol saturation in the bile is increased in the setting of elevated plasma triglycerides, and plasma level of HDL-C was inversely correlated with gallstone prevalence and cholesterol saturation of bile. In the past decade, the effects of *trans*-fatty acids on blood lipid levels have been identified in metabolic studies, which contribute to concern about potential adverse effects of *trans*-fatty acids on risk of gallstone disease. In an earlier study, *trans*-fatty acids at 10% of energy in the diet increased LDL-C and decreased HDL-C levels when substituted for oleic acid.[30] In contrast, when compared with oleic acid, saturated fats increased LDL-C but did not decrease HDL-C levels. The adverse effect of *trans*-fatty acids on the ratio of total cholesterol to HDL-C was approximately twice that of saturated fats. Two other studies also revealed similar results.[31,32] *trans*-Fatty acids also raise plasma triglyceride level, with an increases in triglyceride levels ranging from 1.0 to 24 mg/dL (0.01-0.27 mmol/L).[9,11,33,34]

The prospective design of our study avoids the potential for differential recall of intake by gallstone cases and noncases because all data on food were collected before the diagnosis of gallstone disease. Also, consistently high follow-up rates reduce the possibility that our results are biased by men lost to follow-up in this cohort. Thus, these potential biases should have been minimal.

The possibility of misclassification might be of concern because information on nutrient intake was collected by self-report. Random within-person variation could attenuate any true association of interest, but the SFFQ was designed to minimize this error by assessing average long-term dietary intake during the successive follow-up periods. These repeated measurements took into account possible changes in diet with time and reduced random variation in reporting. Any measurement errors would be expected to be unrelated to the gallstone disease end points because of the prospective design. Thus, any nondifferential misclassification would most likely bias the RRs toward null and weaken any true relationship.

To address the possibility of bias due to latent gallstone disease, we incorporated a lag period of 4 years between dietary assessment at baseline and subsequent development of gallstone disease. The positive association persisted after the first 4 years of follow-up were excluded. In addition, we performed our analysis among men with cholecystectomy and excluded men with un-

removed gallstones who might be presumably less symptomatic and more prone to detection bias. The positive association still persisted after the exclusion.

The long-term effect of *trans*-fatty acid intake on the risk of gallstone disease can only be addressed by epidemiologic means, which could not be adequately addressed by the short-term feeding in the metabolic studies. Although we assessed and adjusted for a number of potential confounders, we cannot exclude the possibility of residual confounding, as in any other observational study. It is possible that the positive association was due to some unmeasured variable, such as socioeconomic status. However, because the population we studied was relatively homogeneous with respect to education and occupation, confounding by socioeconomic status was minimized. More direct data on the relation of intake of *trans*-fatty acids and risk for gallstones might be obtained from randomized trials, but this does not seem to be feasible. Thus, findings from observational studies, as well as from controlled metabolic studies, which indicate potentially adverse effects of these isomers, will be important.

Our results suggest that a higher intake of *trans*-fatty acids modestly increases risk of gallstone disease. This must add to the concern that the practice of partial hydrogenation of vegetable oils to form shortening and margarine can have adverse health effects.[35] Our findings should have implications for additional clinical and mechanistic research.

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